REMARKS/ARGUMENTS

Claims 28-32 are pending in this application.

Applicants acknowledge the Examiner's statement (at pages 29-30 of the Examiner's Answer) that the rejection of Claims 28-32 under 35 U.S.C. §102(a) as allegedly being anticipated by Botstein *et al.* (WO 2000053751) is withdrawn, because the instant application is entitled to an effective filing date of February 18, 2000. The remaining rejections under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph are addressed below.

I. Claim Rejections Under 35 U.S.C. §101 and 35 U.S.C. §112, First Paragraph

Claims 28-32 are rejected under 35 U.S.C. §101 as allegedly lacking either a specific and substantial asserted utility or a well-established utility. Claims 28-32 are further rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement "since the claimed invention is not supported by either a credible, specific and substantial utility or a well established utility ... one skilled in the art clearly would not know how to use the claimed invention." (Page 10 of the Examiner's Answer).

Applicants submit, as discussed below, that not only has the PTO not established a *prima facie* case for lack of utility, but that the antibodies of Claims 28-32 possess a specific and substantial asserted utility, and that based upon this utility, one of skill in the art would know how to use the claimed antibodies without any further experimentation.

The gene amplification data disclosed in Example 143 establishes a credible, substantial and specific patentable utility for the PRO1293 polypeptide and the claimed antibodies that bind it

First of all, Applicants respectfully maintain the position that the specification discloses at least one credible, substantial and specific asserted utility for the PRO1293 polypeptide and the claimed antibodies that bind it for the reasons previously set forth in Applicants' Responses filed on August 19, 2004 and February 28, 2005, and in the Appeal Brief filed November 22, 2005.

Furthermore, as first discussed in Applicants' Response of August 19, 2004, Applicants rely on the gene amplification data for patentable utility of the PRO1293 polypeptide and the claimed antibodies that bind it, and the gene amplification data for the gene encoding the PRO1293 polypeptide is clearly disclosed in the instant specification under Example 143. As

previously discussed, a ΔCt value of at least 1.0 was observed for PRO1293 in at least three of the tumors listed in Table 8. PRO1293 showed approximately 1.71 ΔCt units which corresponds to $2^{1.71}$- fold amplification or 3.27-fold amplification in primary lung tumor (HF-000840), and approximately 1.13-2.33 ΔCt units which corresponds to $2^{1.13}$ -$2^{2.33}$- fold amplification or 2.19 fold to 5.03-fold amplification in colon tumors (HF-000539 and HF-000795). (See Table 8 and page 507, lines 5-12 of the specification).

Accordingly, the present specification clearly discloses overwhelming evidence that the gene encoding the PRO1293 polypeptide is significantly amplified in lung and colon tumors. Thus one of ordinary skill in the art would find it credible that the PRO1293 polypeptide and the claimed antibodies that bind it have utility as diagnostic markers of lung or colon tumors.

The Examiner asserts that the significance of the gene amplification data "can be questioned since 49 out of 52 tested tumor samples did not show an amplification of the gene encoding PRO1293." (Pages 12-13 of the Examiner's Answer). Applicants emphasize that they have shown significant DNA amplification in three of the lung and colon tumor samples in Table 8, Example 143 of the instant specification. The fact that not all lung and colon tumors tested positive in this study does not make the gene amplification data less significant. As any skilled artisan in the field of oncology would easily appreciate, not all tumor markers are generally associated with every tumor, or even with most tumors. For example, the article by Hanna and Mornin (submitted with the Response filed August 19, 2004), discloses that the known breast cancer marker HER-2/neu is "amplified and/or overexpressed in 10%-30% of invasive breast cancers and in 40%-60% of intraductal breast carcinoma" (page 1, col. 1). In fact, some tumor markers are useful for identifying rare malignancies. That is, the association of the tumor marker with a particular type of tumor lesion may be rare, or, the occurrence of that particular kind of tumor lesion itself may be rare. In either event, even these rare tumor markers which do not give a positive hit for most common tumors, have great value in tumor diagnosis, and consequently, in tumor prognosis. The skilled artisan would certainly know that such tumor markers are useful for better classification of tumors. Therefore, whether the PRO1293 gene is amplified in three lung and colon tumors or in all lung and colon tumors is not relevant to its identification as a tumor marker, or its patentable utility. Rather, the fact that the amplification data for PRO1293 is considered significant is what lends support to its usefulness as a tumor marker.

The Examiner further asserts that the gene amplification data are not persuasive because "the control used was not a matched non-tumor lung sample but rather was a pooled DNA sample from the blood of healthy subjects. The art uses matched tissue samples (see Pennica *et al.*)." (Page 13 of the Examiner's Answer).

Applicants respectfully submit that the negative control taught in the specification was known in the art at the time of filing, and accepted as a true negative control as demonstrated by use in peer reviewed publications, including Pennica *et al.* For example, Pennica *et al.* explains that "[t]he relative WISP gene copy number in each colon tumor DNA was compared with **pooled normal DNA** from 10 donors by quantitative PCR" (page 14720, col. 2; emphasis added). Pennica *et al.* further explain that DNA was isolated from "the pooled blood of 10 normal human donors" (page 14718, col. 1). Thus Pennica *et al.* used the **same** control for their gene amplification experiments as that described in the instant specification.

In further examples, Pitti *et al.* (Exhibit F submitted with the Response filed August 19, 2004), used the same quantitative TaqMan PCR assay described in the specification to study gene amplification in lung and colon cancer of DcR3, a decoy receptor for Fas ligand. As described, Pitti *et al.* analyzed DNA copy number "in genomic DNA from 35 primary lung and colon tumours, relative to pooled genomic DNA from peripheral blood leukocytes (PBL) of 10 healthy donors." (Page 701, col. 1; emphasis added). The authors also analyzed mRNA expression of DcR3 in primary tumor tissue sections and found tumor-specific expression, confirming the finding of frequent amplification in tumors, and confirming that the pooled blood sample was a valid negative control for the gene amplification experiments. In Bieche *et al.* (Exhibit G submitted with the Response filed August 19, 2004), the authors used the quantitative TaqMan PCR assay to study gene amplification of *myc*, *ccnd1* and *erbB2* in breast tumors. As their negative control, Bieche *et al.* used normal leukocyte DNA derived from a small subset of the breast cancer patients (page 663). The authors note that "[t]he results of this study are consistent with those reported in the literature" (page 664, col. 2), thus confirming the validity of the negative control. Accordingly, the art demonstrates that pooled normal blood samples are considered to be a valid negative control for gene amplification experiments of the type described in the specification.

The Examiner asserts that "[t]he specification merely demonstrates that the PRO1293 genomic DNA was amplified in some cancers, to a minor degree (about 2-5 fold) relative to normal blood DNA." (Page 13 of the Examiner's Answer).

Applicants respectfully submit that the Examiner seems to be applying a heightened utility standard in this instance, which is legally incorrect. Applicants have shown that the gene encoding PRO1293 demonstrated significant amplification, from 2.19 to 5.03 fold, in three lung and colon tumors. As explained in the Declaration of Dr. Audrey Goddard (submitted with the Response filed August 19, 2004):

> It is further my considered scientific opinion that an at least **2-fold increase** in gene copy number in a tumor tissue sample relative to a normal (*i.e.*, non-tumor) sample **is significant** and useful in that the detected increase in gene copy number in the tumor sample relative to the normal sample serves as a basis for using relative gene copy number as quantitated by the TaqMan PCR technique as a diagnostic marker for the presence or absence of tumor in a tissue sample of unknown pathology. (Emphasis added).

By referring to the 2.19-fold to 5.03-fold amplification of the PRO1293 gene in lung and colon tumors as "minor" the Examiner appears to ignore the teachings within an expert's declaration without any basis, or without presenting any evidence to the contrary. Applicants respectfully draw the Examiner's attention to the Utility Examination Guidelines (Part IIB, 66 Fed. Reg. 1098 (2001)) which state that:

> Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Thus, barring evidence to the contrary, Applicants maintain that the 2.17 to 5.03-fold amplification disclosed for the PRO1293 gene is significant and forms the basis for the utility claimed herein.

The Examiner asserts that the Goddard Declaration is not convincing, because the six references submitted with the Declaration allegedly do not "appear to indicate that an approximately 2-5 fold amplification of genomic DNA is significant in tumors." (Page 13 of the Examiner's Answer). Applicants respectfully submit that this statement is scientifically and factually inaccurate. The three references which discuss applications of the PCR-based gene amplification determination technique to studies of specific genes make clear that values of at

least 2-fold in the assay of Example 143 are considered to meet the threshold for significant amplification in tumors.

In Pennica *et al*, for example, the authors concluded that WISP-1 was aberrantly expressed in human colon tumors based upon an observed amplification of at least 2-fold in about 60% of the tumors tested (page 14720, col. 2). Similarly, in Pitti *et al.*, the authors concluded that DcR3 was amplified in lung and colon tumors based upon an observed amplification ranging from 2 to 18-fold in about half of the tumors tested. In Bieche *et al.*, the authors explicitly state that "**values of 2 or more** were considered to represent gene amplification in tumor DNA" (page 664, col. 1; emphasis added). Thus the art is clear that an observed amplification of at least 2-fold in the assay of Example 143 is considered to be indicative of significant amplification in tumors, sufficient to demonstrate that amplification of the gene is associated with tumors.

Accordingly, Applicants submit that based on the general knowledge in the art at the time the invention was made and the teachings in the specification, the specification provides clear guidance as to how to interpret and use the data relating to PRO1293 expression and that the PRO1293 polypeptide and the claimed antibodies that bind it have utility in the diagnosis of cancer.

A *prima facie* case of lack of utility has not been established

The Examiner has asserted that the disclosed gene amplification data does not establish a patentable utility for the PRO1293 polypeptides because allegedly "it does not necessarily follow that an increase in gene copy (DNA) number results in increased gene expression (mRNA) and increased protein expression such that the polypeptide of SEQ ID NO:77, or variants of the polypeptide of SEQ ID NO:77, would be useful diagnostically." (Pages 5-6 of the Examiner's Answer). In support of the assertion that gene amplification is not correlated with increased mRNA expression, the Examiner refers to Pennica *et al.*, as well as a newly cited reference by Konopka *et al.* (Page 6 of the Examiner's Answer). The Examiner further asserts that "[e]ven if increased mRNA levels could be established for PRO1293, it does not follow that polypeptide levels would also be amplified," referring to Hu *et al.* and a newly cited reference by Chen *et al.* for support. (Pages 6-7 of the Examiner's Answer). Finally, the Examiner asserts that "[t]he art also shows that mRNA (transcript) levels do not correlate with polypeptide levels in normal

tissues, citing five new references by Haynes *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.* and Greenbaum *et al.* (Pages 7-9 of the Examiner's Answer).

As a preliminary matter, Applicants respectfully submit that it is not a legal requirement to establish that gene amplification necessarily results in increased expression at the mRNA and polypeptide levels, or that protein levels can be "accurately predicted." As discussed in Applicants' Appeal Brief, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the Applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. Therefore, it is not legally required that there be a "necessary" correlation between the data presented and the claimed subject matter. The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants respectfully submit that when the proper evidentiary standard is applied, a correlation must be acknowledged.

Pennica *et al.* and Konopka *et al.*

In support of the assertion that gene amplification is not correlated with increased mRNA expression, the Examiner refers to Pennica *et al.*, as well as a newly cited reference by Konopka *et al.* (Page 6 of the Examiner's Answer). In particular, the Examiner cites the abstract of Pennica *et al.* for its disclosure that "WISP-1 gene amplification and overexpression in human colon tumors showed a correlation between DNA amplification and over-expression, whereas overexpression of WISP-3 RNA was seen in the absence of DNA amplification. In contrast, WISP-2 DNA was amplified in colon tumors, but its mRNA expression was significantly reduced in the majority of tumors compared with expression in normal colonic mucosa from the same patient." From this, the Examiner correctly concludes that increased copy number does not *necessarily* result in increased polypeptide expression. The standard, however, is not absolute certainty.

As noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression...*" (Pennica *et al.*, page14722, left column, first full paragraph, emphasis added). Thus the findings of Pennica *et al.* with respect to WISP-1 support Applicants' arguments. In the

case of WISP-3, the authors report that there was no change in the DNA copy number, but there was a change in mRNA levels. This apparent lack of correlation between DNA and mRNA levels is not contrary to Applicants' assertion that a change in DNA copy number generally leads to a change in mRNA level. Applicants are not attempting to predict the DNA copy number based on changes in mRNA level, and Applicants have not asserted that the only means for changing the level of mRNA is to change the DNA copy number. Therefore a change in mRNA without a change in DNA copy number is not contrary to Applicants' assertions.

The fact that the single WISP-2 gene did not show the expected correlation of gene amplification with the level of mRNA/protein expression does not establish that it is more likely than not, in general, that such correlation does not exist. The Examiner has not shown whether the lack or correlation observed for the WISP-2 gene is typical, or is merely a discrepancy, an exception to the rule of correlation. Indeed, the working hypothesis among those skilled in the art is that, if a gene is amplified in cancer, the encoded protein is likely to be expressed at an elevated level, as was demonstrated for WISP-1.

Accordingly, Applicants respectfully submit that Pennica *et al.* teaches nothing conclusive regarding the absence of correlation between amplification of a gene and over-expression of the encoded WISP polypeptide. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

The Examiner argues that Pennica *et al* is relevant even though it is limited to only one gene family because it is "shows a lack of correlation between gene amplification and gene product overexpression" and because the instant case also concerns a single gene. (Page 15 of the Examiner' Answer). Applicants respectfully disagree. The test is whether it is more likely than not that gene amplification results in overexpression of the corresponding mRNA and protein. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does not result in mRNA or protein overexpression. Providing the single example of the WISP-2 gene does not suffice to meet this burden.

Applicants next respectfully submit that, contrary to the PTO's assertions, Konopka *et al.* **supports** Applicants' position that mRNA levels correlate with protein levels. Konopka *et al.* states that "the 8-kb mRNA that encodes $P210^{c\text{-}abl}$ was detected at a 10-fold higher level in SK-

CML7bt-333 (Fig. 3A, +) than in SK-CML16Bt-1 (B, +), which **correlated** with the relative level of $P210^{c\text{-}abl}$ detected in each cell line. Analysis of additional cell lines demonstrated that the level of 8-kb mRNA **directly correlated** with the level of $P210^{c\text{-}abl}$ (Table 1)" (page 4050, col. 2, emphasis added).

Nor does Konopka *et al.* support the PTO's position that DNA amplification is not correlated with mRNA or protein overexpression. Konopka *et al.* show only that, of the cell lines known to have increased abl protein expression, only one had amplification of the abl gene (page 4051, col. 1). This result proves only that increased mRNA and protein expression levels can result from causes other than gene amplification. Konopka *et al.* do not demonstrate that when gene amplification does occur, it does not result in increased mRNA and protein expression levels, particularly given that the cell line with amplification of the abl gene did show increased abl mRNA and protein expression levels.

Hu *et al.* and Chen *et al.*

In support of the assertion that "[e]ven if increased mRNA levels could be established for PRO1293, it does not follow that polypeptide levels would also be amplified," the Examiner refers to Hu *et al.* and a newly cited reference by Chen *et al.* for support. (Pages 6-7 of the Examiner's Answer). In particular, the Examiner cites Hu *et al.* to the effect that genes displaying a 5-fold change or less in mRNA expression in tumors compared to normal showed no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation between expression level and a published role in the disease. (Pages 6-7 of the Examiner's Answer).

Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the Applicant enjoys, the Examiner must establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. Accordingly, contrary to the Examiner's assertion, Applicants submit that Hu *et al.* does not conclusively show that it is more likely than not that gene amplification does not result in increased expression at the mRNA and polypeptide levels.

Applicants respectfully point out that the analysis by Hu *et al.* has certain statistical flaws. According to Hu *et al.*, "*different* statistical methods 'were applied to' *estimate* the

strength of gene-disease relationships and evaluated the results." (See page 406, left column, emphasis added). Using these different statistical methods, Hu *et al.* "[a]ssessed the relative strengths of gene-disease relationships based on the frequency of both co-citation and single citation." (See page 411, left column). It is well known in the art that various statistical methods allow different variables to be manipulated to affect the outcome. For example, the authors admit, "Initial attempts to search the literature using" the list of genes, gene names, gene symbols, and frequently used synonyms, generated by the authors "revealed several sources of false positives and false negatives." (See page 406, right column). The authors further admit that the false positives caused by "duplicative and unrelated meanings for the term" were "difficult to manage." Therefore, in order to minimize such false positives, Hu *et al.* disclose that these terms "had to be eliminated entirely, thereby reducing the false positive rate but unavoidably under-representing some genes." (See page 406, right column). Hence, Applicants respectfully submit that in order to minimize the false positives and negatives in their analysis, Hu *et al.* manipulated various aspects of the input data.

Applicants further submit that the statistical analysis by Hu *et al.* is not a reliable standard because the frequency of citation only reflects the current research interest in a molecule, not the true biological function of the molecule. Indeed, the authors acknowledge that "[r]elationships established by frequency of co-citation do not necessarily represent a true biological link." (See page 411, right column). One would expect that genes with the greatest change in expression in a disease would be the first targets of research, and therefore have the strongest known relationship to the disease as measured by the number of publications reporting a connection with the disease. The correlation reported in Hu only indicates that the greater the change in expression level, the more likely it is that there is a published or known role for the gene in the disease, as found by their automated literature-mining software. Thus, Hu's results merely reflect a bias in the literature toward studying the most prominent targets, and say nothing regarding the ability of a gene that is 2-fold or more differentially expressed in tumors to serve as a disease marker.

Even assuming that Hu *et al.* provide evidence to support a true relationship, the conclusion in Hu *et al.* only applies to a specific type of breast tumor (estrogen receptor (ER)-positive breast tumor) and can not be generalized as a principle governing microarray study of

breast cancer in general, let alone the various other types of cancer genes in general. In fact, even Hu *et al* admit that., "[i]t is likely that this threshold will change depending on the disease as well as the experiment. Interestingly, the observed correlation was only found among ER-positive (breast) tumors not ER-negative tumors." (See page 412, left column). Therefore, based on these findings, the authors add, "[t]his may reflect a bias in the literature to study the more prevalent type of tumor in the population. Furthermore, this emphasizes that caution must be taken when interpreting experiments that may contain subpopulations that behave very differently." (See page 412, left column; emphasis added).

Furthermore, Hu *et al.* did not look for a correlation between changes in mRNA and changes in protein levels, and therefore their results are not contrary to Applicants' assertion that there is a correlation between the two. Applicants are not relying on any "biological role" that the PRO1293 polypeptide has in cancer for its asserted utility. Instead, Applicants are relying on the overexpression of PRO1293 in certain tumors compared to their normal tissue counterparts. Nowhere in Hu does it say that a lack of correlation in their study means that genes with a less than five-fold change in level of expression in cancer cannot serve as a diagnostic marker of cancer.

The Examiner asserts that "Appellant is holding Hu *et al.* to a higher standard than their own specification" for statistical analysis. (Page 17 of the Examiner's Answer). However, Applicants have compared the level of amplification of the PRO1293 gene in normal tissue and lung and colon tumors and have provided information indicating a greater than 2-fold amplification. Applicants are not relying on statistical analysis of information obtained from published literature based on the current research interest of a molecule, and hence the issues regarding statistical analysis of such information do not apply to Applicants' data.

The Examiner further cites a new reference by Chen *et al.* as allegedly disclosing that "only 17% of 165 polypeptide spots or 21% of the genes had a significant correlation between protein and mRNA expression levels" in lung adenocarcinoma samples. (Page 6 of the Examiner's Answer).

First, Applicants note that proteins selected for study by Chen *et al.* were those detectable by staining of 2D gels. As noted in, for example, Haynes *et al.*, cited by the Examiner in the Examiner's Answer, there are problems with selecting proteins detectable by 2D gels. "It is

apparent that without prior enrichment only a relatively small and highly selected population of long-lived, highly expressed proteins is observed. There are many more proteins in a given cell which are not visualized by such methods. Frequently it is the low abundance proteins that execute key regulatory functions." (page 1870, col. 1). Thus, Chen *et al.*, by selecting proteins detectable by staining of 2D gels, are likely to have excluded from their analysis many of the proteins most likely to be significant as cancer markers.

Secondly, Chen *et al.* looked at expression levels across a set of samples including a large number of tumor samples (76) along with a much smaller number of normal samples (9). The tumor samples were taken from stage 1 and stage III lung adenocarcinomas, which were classified as bronchoaveolar, bronchial derived or both bronchial and bronchoaveolar derived. Accordingly, the tissues examined were from different tissues in different stages of normal or cancerous growth. The authors determined the relationship between mRNA and protein expression by using the average expression values for all samples. The average value for each protein or mRNA was generated using all 85 lung tissue samples. This resulted in negative normalized protein values in some cases. Further, the authors chose an arbitrary threshold of 0.115 for the correlation to be considered significant. Accordingly, the Chen paper does not account for different expression in different tissues or different stages of cancer.

Thirdly, no attempt was made to compare expression levels in normal versus tumor samples, and in fact the authors concede that they had too few normal samples for meaningful analysis (page 310, col. 2). As a result, the analysis in the Chen paper shows only that a number of randomly selected proteins have varying degrees of correlation between mRNA and protein expression levels within a set of different lung adenocarcinoma samples. The Chen paper does not address the issue of whether increased mRNA levels in the tumor samples taken together as one group, as compared to the normal samples as a group, correlated with increased protein levels in tumorous versus normal tissue. Accordingly, the results presented in the Chen paper are not applicable to the application at issue.

The correct test of utility is whether the utility is "more likely than not". In the case of the Chen reference, even if the analysis presented is correct (which is disputed), a review of the correlation coefficient data presented in the Chen *et al.* paper indicates that it is more likely than not that increased mRNA expression correlates with increased protein expression. A review of

Table 1, which lists 66 genes [the paper incorrectly states there are 69 genes listed] for which only one protein isoform is expressed, shows that 40 genes out of 66 had a positive correlation between mRNA expression and protein expression. This clearly meets the test of "more likely than not." Similarly, in Table II , 30 genes with multiple isoforms [again the paper incorrectly states there are 29] were presented. In this case, for 22 genes out of 30, at least one isoform showed a positive correlation between mRNA expression and protein expression. Furthermore, 12 genes out of 29 showed a strong positive correlation [as determined by the authors] for at least one isoform. No genes showed a significant negative correlation. It is not surprising that not all isoforms are positively correlated with mRNA expression. Certain isoforms are likely non-functional proteins. Thus, Table II also provides that it is more likely than not that protein levels will correlate with mRNA expression levels.

The same authors in Chen *et al.*, published a later paper, Beer *et al.*, Nature Medicine 8(8) 816-824 (2002) (copy enclosed) which described gene expression of genes in adenocarcinomas and compared that to protein expression. In this paper they report that "these results suggest that the oligonucleotide microarrays provided reliable measures of gene expression" (page 817). The authors also state, "these studies indicate that many of the genes identified using gene expression profiles are likely relevant to lung adenocarcinoma." Clearly the authors of the Chen paper agree that microarrays provide a reliable measure of the expression levels of the gene and can be used to identify genes whose overexpression is associated with tumors.

Haynes *et al.* and Gygi *et al.*

The Examiner cites a new reference by Haynes *et al.* in support of the assertion that "mRNA (transcript) levels do not correlate with polypeptide levels in normal tissues." (Page 7 of the Examiner's Answer). Applicants respectfully point out that Haynes *et al.* never indicate that the correlation between mRNA and protein levels does not exist. Haynes *et al.* only state that "protein levels cannot be *accurately* predicted from the level of the corresponding mRNA transcript" (See page 1863, under Section 2.1, last line, emphasis added). This result is expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, Haynes *et al.* concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA

transcript in a single cellular stage or type when looking at the level of transcripts across different genes.

Importantly, Haynes *et al.* did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing the level of mRNA for a particular gene leads to a corresponding increase for the encoded protein. Haynes *et al.* did not study this issue and says absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA for a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes *et al.* is not inconsistent with or contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

Furthermore, Applicants note that contrary to the Examiner's statement, Haynes teaches that "*there was a general trend* but no strong correlation between protein [expression] and transcript levels" (See page 1863, under Section 2.1, emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied but the correlation is not linear, hence the authors suggest that one cannot *accurately* predict protein levels from mRNA levels. In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Haynes *et al.*

Haynes *et al.* may teach that protein levels cannot be "accurately predicted" from mRNA levels in the sense that the exact numerical amounts of protein present in a tissue cannot be determined based upon mRNA levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is relative levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such

that mRNA overexpression generally predict protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

The Examiner also cites a new reference by Gygi *et al.*, a study on which the Haynes references is based. (Page 7 of the Examiner's Answer). Like Haynes, the Gygi reference looked at levels of mRNA at the same growth phase across different genes, not changes in mRNA levels for a single gene. Thus, when Gygi *et al.* state that "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data," the authors are referring to correlations between constant levels of mRNA and protein at the same growth phase across different genes, not a correlation between a change in mRNA level and a change in protein level for the same gene and corresponding protein. Therefore, for the same reasons that Haynes is not relevant to Applicants' asserted utility, Gygi likewise offers no support for the PTO's rejection of Applicants' asserted utility.

Applicants further submit that Gygi *et al* did not indicate that a correlation between mRNA and protein levels does not exist. Gygi *et al.* only state that the correlation may not be sufficient in **accurately** predicting protein level from the level of the corresponding mRNA transcript (Emphasis added) (see page 1270, Abstract). *Accurate prediction* is not a criteria that is necessary for meeting the utility standards. Applicants note that the Gygi data indicate **a general trend** of correlation between protein [expression] and transcript levels (Emphasis added). For example, as shown in Figure 5, an mRNA abundance of **250-300** copies /cell correlates with a protein abundance of **500-1000** x 10^3 copies/cell. An mRNA abundance of **100-200** copies/cell correlates with a protein abundance of **250-500** x 10^3 copies/cell (emphasis added). Therefore, high levels of mRNA **generally** correlate with high levels of proteins. In fact, most data points in Figure 5 did not deviate or scatter away from the general trend of correlation. Thus, the Gygi data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Gygi *et al.*

Furthermore, Applicants respectfully submit that Futcher *et al.* (Mol Cell Biol. 19: 7357-68 (1999); copy enclosed as Exhibit 13) completed a subsequent study that generated similar data, but reached different conclusions. Futcher *et al.* point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." In

particular, Futcher *et al.* note that Gygi *et al.* used an inappropriate correlation coefficient in the analysis of their data. When the correct statistical methods were applied to the data of Gygi *et al.*, a good correlation was seen.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots.

In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "**the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo***" (page 7367, col. 2; emphasis added).

Lian *et al.*

In further support of the alleged lack of correlation between mRNA expression and protein expression levels, the PTO has cited Lian *et al.* for the statement that there is a poor correlation between mRNA expression and protein abundance in mouse cells, and therefore it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels. (Page 8 of the Examiner's Answer).

In Lian *et al.*, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization to examine the expression of genes at 0, 24, 48 and 72 hours after treatment with

retinoic acid. Protein levels were qualitatively assessed at 0 and 72 hours after retinoic acid treatment following 2-dimensional gel electrophoresis.

Lian *et al.* report that they were able to identify 28 proteins which they considered differentially expressed (page 521). Of those 28, only 18 had corresponding gene expression information, and only 13 had measurable levels of mRNA expression (page 521, Table 6). The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels (page 521, col. 1). The authors note that "[n]one of these was on the list of genes that were differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)" (page 521; emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in 2DE, there was poor correlation between changes in mRNA levels and estimated protein levels" (page 522, col. 2).

The authors themselves admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and proteins presented at low level will be missed. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this decreased the sensitivity of detection of minor proteins." (emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable. In particular, the total number of proteins examined by Lian *et al.* was only 50 (page 520, col. 2), as compared to the approximately 7000 genes for which mRNA levels were measured (page 515, col. 1). Thus the conclusions are based on a very small and atypical set of proteins.

Applicants also emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. As discussed above, Lian *et al.* did not take genes which showed significant mRNA changes and check the

corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, for which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between a change in mRNA level and a corresponding change in protein level. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. Thus, there is little basis for the authors' conclusion that "it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels (as estimated from 2DE)."

Finally, Applicants submit that Lian *et al.* only teach that protein expression may not correlate with mRNA level in differentiating myeloid cells and does not teach anything regarding such a lack of correlation for genes in general. Myeloid cell differentiation relates to hematopoiesis and is an entirely different biological process from solid tumor development because these two process involve entirely different regulatory mechanisms and molecules. Analysis of surface antigens expressed on myeloid cells of the granulocyte-monocyte-histiocyte series during differentiation in normal and malignant myelomonocytic cells is useful in identifying and classifying human leukemias and lymphomas, but cannot be used in diagnosis of any solid tumors. Therefore, even if the teaching of Lian *et al.* accurately reflects the correlation

between mRNA and protein for the particular system studied, it can not apply to the tumor diagnosis assays of the present application.

Fessler *et al.*

The Examiner also cites a publication by Fessler *et al.*, as having "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." (Page 8 of the Examiner's Answer). Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. In Table VIII, Fessler *et al.* list a comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of the 13 up-regulated proteins, a change in mRNA levels is reported for only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Of the 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Regarding the remainder of the proteins listed in Table VIII, in 6 instances, protein levels changed while mRNA levels were unchanged. This evidence has no relevance to Applicants' assertion that changes in mRNA levels lead to corresponding changes in protein levels, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that changes in mRNA levels causes corresponding changes in protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased, decreased or remained unchanged in PMN upon contact with LPS. Nothing in these results by Fessler *et al.* suggests that a change in

the level of mRNA for a particular protein does not generally lead to a corresponding change in the level of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

The PTO points to Fessler's statement regarding Table VIII that there was "a poor concordance between mRNA transcript and protein expression changes." (Page 8 of the Examiner's Answer). As is clear from the above discussion, this statement does not relate to a lack of correlation between a change in mRNA levels leading to a change in protein levels, because in 5 of 6 such instances, changes in mRNA and protein levels correlated well. Instead, this statement relates to observations in which protein levels changed when mRNA was either unchanged or "absent." As such, this statement is an observation that in addition to transcriptional activity, LPS also has post-transcriptional and possibly post-translational activity that affect protein levels, an observation which is not contrary to Applicants' assertions. Accordingly, Fessler's results are consistent with Applicants' assertion that a change in mRNA level of for a particular protein generally leads to a corresponding change in the level of the encoded protein, since 5 of 6 genes demonstrated such a correlation.

Greenbaum *et al.*

In further support of the alleged lack of correlation between mRNA expression and protein expression levels, the Examiner cites an additional new reference by Greenbaum *et al.* The Examiner asserts that Greenbaum *et al.* teaches that, "To date, there have been only a handful of efforts to find correlations between mRNA and protein expression levels... And, for the most part, they have reported only minimal and/or limited correlations." (Page 8 of the Examiner's Answer).

Applicants note that Greenbaum *et al.* compared the expression of a number of different mRNAs and their corresponding proteins in yeast cells. Greenbaum *et al.* did not compare the change of expression of specific mRNAs and their corresponding proteins in cancer cells versus normal cells. Accordingly, this reference is also not relevant to the issue at hand. Nevertheless, Greenbaum states that logically "we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level. The variability of the mRNA expression is indicative of the cell controlling the mRNA expression at different points of the cell cycle to achieve the resulting and desired protein. **Thus we would expect and we found**

a high degree of correlation (r-0.89) between the reference mRNA and protein levels for these particular ORFs: the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression" (page 117.5, col. 1; emphasis added). Furthermore, Greenbaum states that "we found that ORFs that have higher than average levels of ribosomal occupancy – that is that a large percentage of their cellular mRNA concentration is associated with ribosomes (being translated) – have well correlated mRNA and protein expression levels. (Figure 2)." (page 117.5, col. 2; emphasis added). Therefore, contrary to the Examiner's assertion, Greenbaum does find high levels of correlation between mRNA and protein expression in yeast cells. In particular, Greenbaum demonstrates that a high degree of correlation is found for those genes which show a large degree of variability in mRNA expression – that is, for those genes which show changes in mRNA expression, the change in mRNA expression is correlated with a change in protein expression.

In summary, Applicants respectfully submit that the Examiner has not shown that gene amplification in tumor as compared to normal tissue is not correlated with changes in mRNA and protein expression. The Patent Office has failed to meet its initial burden of proof that Applicants' claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the Pennica, Konopka, Hu, Chen, Haynes, Gygi, Lian, Fessler, and Greenbaum articles do not provide sufficient reasons to doubt the statements by Applicants that PRO1293 has utility. As discussed above, the law does not require the existence of a "necessary" correlation between gene amplification and mRNA and protein expression levels. Nor does the law require that protein levels be "accurately predicted." According to the authors themselves, the data in the above cited references confirm that there is a general trend between gene amplification and mRNA and protein expression levels, which meets the "more likely than not standard" and show that a positive correlation exists between gene amplification and mRNA and protein expression. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is overexpressed in cancer, it is more likely than not that the encoded protein will also be expressed at an elevated level.

It is "more likely than not" for amplified genes to have increased mRNA and protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed August 19, 2004) collectively teach that in general, gene amplification increases mRNA expression. Second, the Declaration of Dr. Paul Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, shows that, in general, there is a correlation between mRNA levels and polypeptide levels.

The Examiner has asserted that "Orntoft et al. could only compare the levels of about 40 well-resolved and focused abundant proteins." (Page 18 of the Examiner's Answer). Applicants respectfully point out that while technical considerations did prevent Orntoft *et al.* from evaluating a larger number of proteins, the ones they did look at showed a clear correlation between mRNA and protein expression levels. The authors found that "[i]n general **there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations**. Only one gene [of the 40 examined] showed disagreement between transcript alteration and protein alteration" (page 42, col. 2; emphasis added). Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

The Examiner further asserts that "Applicants have provided no fact or evidence concerning a lack of correlation between the specification's disclosure of low levels of amplification of DNA (which were not characterized on the basis of those in the Orntoft publication) and an associated rise in level of the encoded protein." (Page 18 of the Examiner's Answer).

As discussed above, the levels of amplification for PRO1293 were **not** "low" but significant, and ranged from 2.19-fold to 5.03-fold, in three different lung and colon tumors. Applicants note that the levels of gene amplification observed by Orntoft *et al.* were relatively low, averaging only 0.3-0.4-fold (page 40, col. 1). In particular, the level of gene amplification associated with expression changes was only around two-fold (see Figure 2), even less than the

2.19-fold to 5.03-fold amplification observed for PRO1293. Even with these relatively low levels of gene amplification, Orntoft *et al.* found that "**[i]n most cases**, chromosomal gains detected by CGH were accompanied by an increased level of transcripts in both TCCs 733 (77%) and 827 (80%)" (page 40, col. 2; emphasis added). The level of correlation between DNA copy number and increased mRNA levels observed by Orntoft *et al.*, from **77-80%**, clearly meets the standard of more likely than not. Orntoft *et al.* also found a "highly significant" correlation between mRNA and protein levels, with the two data sets studied having correlations of 39/40 (**98%**) and 19/26 (**73%**) (pages 42-43).

The Examiner also states that Orntoft *et al.* do not compare gene expression in cancerous versus non-cancerous tissue, and thus "Orntoft *et al.* did not find any cancer markers." (Page 21 of the Examiner's Answer). Applicants note that while Orntoft *et al.* did not compare cancerous versus non-cancerous tissues, they did compare invasive versus benign tumors, thus finding genes that were markers of tumor malignancy.

Applicants respectfully submit that the Examiner also appears to misunderstand the data presented by Hyman *et al.* The Examiner asserts that "of the 12,000 transcripts analyzed, a set of 270 was identified in which overexpression was attributable to gene amplification." The Examiner concludes that "[t]his proportion is 2%; the Examiner maintains that 2% does not provide a reasonable expectation that the slight amplification of PRO1293 would be correlated with elevated levels of mRNA." (Page 18 of the Examiner's Answer). Applicants respectfully submit that the Examiner appears to have misinterpreted the results of Hyman *et al.* Hyman *et al.* chose to do a genome-wide analysis of a large number of genes, most of which, as shown in Figure 2, were not amplified. Accordingly, the 2% number is meaningless, as the low figure mainly results from the fact that only a small percentage of genes are amplified in the first place. The significant figure is not the percentage of genes in the genome that show amplification, but the percentage of amplified genes that demonstrate increased mRNA and protein expression.

The Examiner further asserts that the Hyman reference "found 44% of *highly* amplified genes showing overexpression at the mRNA level, and 10.5% of highly overexpressed genes being amplified; thus, even at the level of high amplification and high overexpression, the two do not correlate." (Page 18 of the Examiner's Answer). Applicants submit that the 10.5% figure is not relevant to the issue at hand. One of skill in the art would understand that there can be more

than one cause of overexpression. The issue is not whether overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression.

The Examiner's assertion is not consistent with the interpretation Hyman *et al.* themselves place on their data, stating that, "The results illustrate **a considerable influence of copy number on gene expression patterns**." (page 6242. col. 1; emphasis added). In the more detailed discussion of their results, Hyman *et al.* teach that "[u]p to 44% of the highly amplified transcripts (CGH ratio, >2.5) were overexpressed (***i.e.*, belonged to the global upper 7% of expression ratios**) compared with only 6% for genes with normal copy number." (See page 6242, col. 1; emphasis added). These details make it clear that Hyman *et al.* set a highly restrictive standard for considering a gene to be overexpressed; yet almost half of all highly amplified transcripts met even this highly restrictive standard. Therefore, the analysis performed by Hyman *et al.* clearly shows that it is "more likely than not" that a gene which is amplified in tumor cells will have increased gene expression.

The Examiner asserts that Hyman *et al.* and Pollack *et al.* do not examine protein expression. (Page 18 of the Examiner's Answer). Applicants submit that the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* were submitted primarily as evidence that in general, gene amplification increases mRNA expression. As evidence that, in general, there is a correlation between mRNA levels and polypeptide levels, Applicants further submitted the Declaration of Dr. Paul Polakis. Thus Applicants do not rely upon the Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* articles to show a correlation between mRNA levels and polypeptide levels, because such a correlation is demonstrated in the Polakis Declaration. Nonetheless, as discussed above, Orntoft *et al.* does provide evidence that increased mRNA levels in tumor cells are associated with increased protein levels in the same tumor cells.

Finally, the Examiner asserts that "Pollack *et al.* is similarly limited to highly amplified genes which were not evaluated by the method of the instant specification." The Examiner further notes that none of the three references is directed to lung or colon cancer. (Page 18 of the Examiner's Answer). Applicants note that, as discussed above, the levels of amplification for PRO1293 were **not** "low" but **significant**. Applicants further respectfully submit that the Examiner has provided no arguments or evidence as to why the data from Orntoft *et al.*, Hyman

et al. and Pollack *et al.*, concerning gene expression in bladder and breast tumors, would not also apply to tumors in general.

With regard to the correlation between mRNA expression and protein levels, the Examiner has asserted that the Polakis Declaration is insufficient to overcome the rejection of the claims since it is limited to a discussion of data regarding the correlation of mRNA levels and polypeptide levels and not gene amplification levels. The Examiner further asserts that there is "strong opposing evidence showing that gene amplification is not predictive of increased mRNA levels in normal tissues and, in turn, that increased mRNA levels are frequently not predictive of increased polypeptide levels." (Pages 19-20 of the Examiner's Answer).

Applicants submit that Dr. Polakis' Declaration was presented to support the position that there is a correlation between mRNA levels and polypeptide levels, the correlation between gene amplification and mRNA levels having already been established by the data shown in the Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* articles. With regard to the alleged "strong opposing evidence" that increased mRNA levels are not predictive of increased polypeptide levels, Applicants have discussed in detail above the reasons why the data in the Hu, Chen, Haynes, Gygi, Lian, Fessler, and Greenbaum articles confirm that there is a general trend between mRNA and protein expression levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA expression and protein expression.

The Examiner asserts that "the data are not included in the declaration so that the examiner could not independently evaluate them." (Page 20 of the Examiner's Answer). Applicants emphasize that the opinions expressed in the Polakis Declaration are all based on factual findings. Thus, Dr. Polakis explains that in the course of their research using microarray analysis, he and his co-workers identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. Subsequently, antibodies binding to about 30 of these tumor antigens were prepared, and mRNA and protein levels were compared. In approximately 80% of the cases, the researchers found that increases in the level of a particular mRNA correlated with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells. Dr. Polakis' statement that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the

tumor cell relative to the normal cell" is based on factual, experimental findings, clearly set forth in the Declaration. Accordingly, the Declaration is not merely conclusive, and the fact-based conclusions of Dr. Polakis would be considered reasonable and accurate by one skilled in the art.

Furthermore, without acquiescing to the propriety of this rejection, and merely to expedite prosecution in this case, **Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B**. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument."[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner."[3] Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." The statement in question from an expert in the field (the Polakis Declaration) states: "it is my

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[1] *In re Rinehart,* 531 F.2d 1084, 189 U.S.P.Q. 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F2d. 1015, 226 U.S.P.Q. 881 (Fed. Cir. 1985).

[2] *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir 1996) (quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)).

[3] *Id.* at 1583.

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell." Therefore, barring evidence to the contrary regarding the above statement in the Polakis declaration, this rejection is improper under both the case law and the Utility guidelines.

Both Polakis Declarations (Polakis I and II) are further supported by the teachings in Molecular Biology of the Cell, a leading textbook in the field (Bruce Alberts, *et al.*, Molecular Biology of the Cell (3rd ed. 1994) (copy enclosed, herein after Cell 3rd) and (4th ed. 2002) (copy enclosed, herein after Cell 4th). Figure 9-2 of Cell 3rd shows the steps at which eukaryotic gene expression can be controlled. The first step depicted is transcriptional control. Cell 3rd provides that "[f]or most genes transcriptional controls are paramount. This makes sense because, of all the possible control points illustrated in Figure 9-2, only transcriptional control ensures that no superfluous intermediates are synthesized." Cell 3rd at 403 (emphasis added). In addition, the text states that "Although controls on the initiation of gene transcription are the predominant form of regulation for most genes, other controls can act later in the pathway from RNA to protein to modulate the amount of gene product that is made." Cell 3rd at 453 (emphasis added). Thus, as established in Cell 3rd, the predominant mechanism for regulating the amount of protein produced is by regulating transcription initiation.

In Cell 4th, Figure 6-3 on page 302 illustrates the basic principle that there is a correlation between increased gene expression and increased protein expression. The accompanying text states that "a cell can change (or regulate) the expression of each of its genes according to the needs of the moment – *most obviously by controlling the production of its mRNA*." Cell 4th at 302 (Emphasis added). Similarly, Figure 6-90 on page 364 of Cell 4th illustrates the path from gene to protein. The accompanying text states that while potentially each step can be regulated by the cell, "the initiation of transcription is the most common point for a cell to regulate the expression of each of its genes." Cell 4th at 364 (Emphasis added). This point is repeated on page 379, where the authors state that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (Emphasis added).

Further support for Applicants' position can be found in the textbook, Genes VI, (Benjamin Lewin, Genes VI (1997)) (copy enclosed) which states "having acknowledged that

control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added).

Additional support is also found in Zhigang *et al.*, World Journal of Surgical Oncology 2:13, 2004 (copy enclosed). Zhigang studied the expression of prostate stem cell antigen (PSCA) protein and mRNA to validate it as a potential molecular target for diagnosis and treatment of human prostate cancer. The data showed "a high degree of correlation between PSCA protein and mRNA expression" *Zhigang* at 4. Of the samples tested, 81 out of 87 showed a high degree of correlation between mRNA expression and protein expression. The authors conclude that "it is demonstrated that PSCA protein and mRNA overexpressed in human prostate cancer, and that the increased protein level of PSCA resulted from the upregulated transcription of its mRNA." *Zhigang* at 6. Even though the correlation between mRNA expression and protein expression occurred in 93% of the samples tested, not 100%, the authors state that "PSCA may be a promising molecular marker for the clinical prognosis of human Pca and a valuable target for diagnosis and therapy of this tumor." *Id.* at 7.

Further, Meric *et al.*, Molecular Cancer Therapeutics, vol. 1, 971-979 (2002) (copy enclosed) states the following:

> The **fundamental principle** of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells...[M]ost efforts have concentrated on identifying differences in gene expression at the level of mRNA, which can be attributable to either DNA amplification or to differences in transcription. Meric *et al.* at 971 (emphasis added).

Those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression.

Together, the Declarations of Polakis, the accompanying references, and the excerpts and references provided above all establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

In addition to the supporting references previously submitted by Applicants, Applicants submit the following references to further support the assertion that changes in mRNA levels generally lead to corresponding changes in the level of the encoded polypeptide.

In a study by Wang *et al.* (Urol. Res. 2000; 28(5):308-15) (attached as Exhibit 3) the authors report that down-regulation of E-cadherin protein has been shown in various human

tumors. *Id.* at Abstract. In the reported study, the authors examined the expression of cadherins and associated catenins at the mRNA level in paired tumor and non-neoplastic primary prostate cultures. They report that "[s]ix of seven cases of neoplastic cultures showed moderately-to-markedly decreased levels of E-cadherin and P-cadherin mRNA. Similar losses of alpha-catenin and beta-catenin mRNA were also observed." *Id.* As Applicants' assertion would predict, the authors state that the mRNA measures showed "good correlation" with the results from protein measures. The authors conclude by stating that "this paper presents a coordinated down-regulation in the expression of E-cadherin and associated catenins at the mRNA and protein level in most of the cases studied." *Id.*

In a more recent study by Munaut *et al.* (Int. J. Cancer. 2003; 106(6):848-55) (attached as Exhibit 4) the authors report that vascular endothelial growth factor (VEGF) is expressed in 64-95% of glioblastomas (GBMs), and that VEGF receptors (VEGFR-1, its soluble form sVEGFR-1, VEGFR-2 and neuropilin-1) are expressed predominantly by endothelial cells. *Id.* at Abstract. The authors explain that infiltrating tumor cells and newly-formed capillaries progress through the extracellular matrix by local proteolysis involving matrix metalloproteinases (MMPs). In the present study, the authors "used quantitative RT-PCR, Western blot, gelatin zymography and immunohistochemistry to study the expression of VEGF, VEGFR-1, VEGFR-2, sVEGFR-1, neuropilin-1, MT1-MMP, MMP-2, MMP-9 and TIMP-2 in 20 human GBMs and 5 normal brains. The expression of these MMPs was markedly increased in most GBMs with excellent correlation between mRNA and protein levels." *Id.* Thus, the results support Applicants' assertion that changes in mRNA level lead to corresponding changes in protein level.

In another recent study, Hui *et al.* (Leuk. Lymphoma. 2003; 44(8):1385-94 (abstract attached as Exhibit 5) used real-time quantitative PCR and immunohistochemistry to evaluate cyclin D1 mRNA and protein expression levels in mantle cell lymphoma (MCL). *Id.* at Abstract. The authors report that seven of nine cases of possible MCL showed overexpression of cyclin D1 mRNA, while two cases showed no cyclin D1 mRNA increase. *Id.* Similarly, "[s]ix of the seven cyclin D1 mRNA overexpressing cases showed increased cyclin D1 protein on tissue array immunohistochemistry; one was technically suboptimal." *Id.* The authors conclude that the study "demonstrates good correlation and comparability between measure of cyclin D1 mRNA ... and cyclin D1 protein." *Id.* Thus, this reference supports Applicants' assertion.

In a recent study by Khal *et al.* (Int. J. Biochem. Cell Biol. 2005; 37(10):2196-206) (abstract attached as Exhibit 6) the authors report that atrophy of skeletal muscle is common in patients with cancer and results in increased morbidity and mortality. *Id.* at Abstract. To further understand the underlying mechanism, the authors studied the expression of the ubiquitin-proteasome pathway in cancer patient muscle using a competitive RT-PCR to measure expression of mRNA for proteasome subunits C2 and C5, while protein expression was determined by western blotting. "Overall, both C2 and C5 gene expression was increased by about three-fold in skeletal muscle of cachectic cancer patients (average weight loss 14.5+/-2.5%), compared with that in patients without weight loss, with or without cancer. ... There was a good correlation between expression of proteasome 20Salpha subunits, detected by western blotting, and C2 and C5 mRNA, showing that increased gene expression resulted in increased protein synthesis." These findings support Applicants' assertion that changes in mRNA level lead to changes in protein level.

Maruyama *et al.* (Am. J. Patho. 1999; 155(3):815-22) (attached as Exhibit 7) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that pancreatic cancer cell lines frequently coexpressed all three Ids, "exhibiting good correlation between Id mRNA and protein levels." *Id.* at Abstract. In addition, the authors teach that all three Id mRNA levels were expressed at high levels in pancreatic cancer samples compared to normal or CP samples. At the protein level, Id-1 and Id-2 staining was faint in normal tissue, while Id-3 ranged from weak to strong. In contrast, in the cancer tissues "many of the cancer cells exhibited abundant Id-1, Id-2, and Id-3 immunoreactivity," and Id-1 and Id-2 protein was increased significantly in the cancer cells by comparison to the respective controls, mirroring the overexpression at the mRNA level. Thus, the authors report that in both cell lines and tissue samples, increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Support for Applicants' assertion is also found in an article by Caberlotto *et al.* (Neurosci. Lett. 1999; 256(3):191-4) (abstract attached as Exhibit 8). In a previous study, the authors investigated alterations of neuropeptide Y (NPY) mRNA expression in the Flinders Sensitive Line rats (FSL), an animal model of depression. *Id.* at Abstract. The authors reported that in the current study, that NPY-like immunoreactivity (NPY-LI) was decreased in the

hippocampal CA region, and increased in the arcuate nucleus, and that fluoxetine treatment elevated NPY-LI in the arcuate and anterior cingulate cortex. The authors state that "[t]he results demonstrate a good correlation between NPY peptide and mRNA expression." Thus, increases and decreases in mRNA levels were reflected in corresponding changes in protein level.

Misrachi and Shemesh (Biol. Reprod. 1999; 61(3):776-84) (abstract attached as Exhibit 9) investigated their hypothesis that FSH regulates the bovine cervical prostaglandin E(2) (PGE(2)) synthesis that is known to be associated with cervical relaxation and opening at the time of estrus. *Id.* at Abstract. Cervical tissue from pre-estrous/estrous, luteal, and postovulatory cows were examined for the presence of bovine (b) FSH receptor (R) and its corresponding mRNA. The authors report that bFSHR mRNA in the cervix was maximal during pre-estrus/estrus, and that the level of FSHR protein was significantly higher in pre-estrous/estrous cervix than in other cervical tissues. *Id.* The authors state that "[t]here was a good correlation between the 75-kDa protein expression and its corresponding transcript of 2.55 kb throughout the estrous cycle as described by Northern blot analysis as well as RT-PCR." *Id.* Thus, changes in the level of mRNA for bFSHR led to corresponding changes in FSHR protein levels, a result which supports Applicants' assertion.

In a study by Stein *et al.* (J. Urol. 2000; 164(3 Pt 2):1026-30) (abstract attached as Exhibit 10), the authors studied the role of the regulation of calcium ion homeostasis in smooth muscle contractility. *Id.* at Abstract. The authors investigated the correlation between sarcoplasmic endoplasmic reticulum, calcium, magnesium, adenosine triphosphatase (SERCA) protein and gene expression, and the contractile properties in the same bladder. Partial bladder outlet obstructions were created in adult New Zealand white rabbits, which were divided into control, sham operated and obstructed groups. Stein *et al.* report that "[t]he relative intensities of signals for the Western [protein] and Northern [mRNA] blots demonstrated a strong correlation between protein and gene expression. ... The loss of SERCA protein expression is mediated by down-regulation in gene expression in the same bladder." *Id.* This report supports Applicants' assertion that changes in mRNA level, e.g. a decrease, lead to a corresponding change in the level of the encoded protein, *e.g.*, a decrease.

In an article by Gou and Xie (Zhonghua Jie He He Hu Xi Za Zhi. 2002; 25(6):337-40) (abstract attached as Exhibit 11) the authors investigated the expression of macrophage migration

inhibitory factor (MIF) in human acute respiratory distress syndrome(ARDS) by examining the expression of MIF mRNA and protein in lung tissue in ARDS and normal persons. *Id.* at Abstract. The authors report "undetectable or weak MIF mRNA and protein expression in normal lungs. In contrast, there was marked upregulation of MIF mRNA and protein expression in the ARDS lungs." *Id.* This is consistent with Applicants' assertion that a change in mRNA for a particular gene, *e.g.*, an increase, generally leads to a corresponding change in the level of protein expression, *e.g.*, an increase.

These studies are representative of numerous published studies which support Applicants' assertion that changes in mRNA level generally lead to corresponding changes in the level of the expressed protein. Applicants submit herewith an addition 70 references (abstracts attached as Exhibit 12) which support Applicants' assertion.

In addition to these supporting references, Applicants also submit herewith additional references which offer support of Applicants' asserted utility by showing that, in general, mRNA expression levels correlate with protein expression levels.

For example, in an article by Futcher *et al.* (Mol. Cell Biol. 1999; 19(11):7357-68) (attached as Exhibit 13) the authors conducted a study of mRNA and protein expression in yeast. Futcher *et al.* report "a good correlation between protein abundance, mRNA abundance, and codon bias." *Id.* at Abstract.

In a study which is more closely related to Applicants' asserted utility, Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied." *Id.* Thus, in these cancer cell lines, DDX1 mRNA and protein levels are correlated.

Similarly, in an article by Papotti *et al.* (Virchows Arch. 2002; 440(5):461-75) (abstract attached as Exhibit 15) the authors examined the expression of three somatostatin receptors (SSTR) at the mRNA and protein level in forty-six tumors. *Id.* at Abstract. The authors report a "good correlation between RT-PCR [mRNA level] and IHC [protein level] data on SSTR types 2, 3, and 5." *Id.*

Van der Wilt *et al.* (Eur. J. Cancer. 2003; 39(5):691-7) (abstract attached as Exhibit 16) studied deoxycytidine kinase (dCK) in seven cell lines, sixteen acute myeloid leukemia samples, ten human liver samples, and eleven human liver metastases of colorectal cancer origin. *Id.* at Abstract. The authors report that "enzyme activity and protein expression levels of dCK in cell lines were closely related to the mRNA expression levels" and that there was a "good correlation between the different dCK measurements in malignant cells and tumors." *Id.*

Grenback *et al.* (Regul. Pept. 2004; 117(2):127-39) (abstract attached as Exhibit 17) studied the level of galanin in human pituitary adenomas using a specific radioimmunoassay. *Id.* at Abstract. The authors report that "[i]n the tumors analyzed with in situ hybridization there was a good correlation between galanin peptide levels and galanin mRNA expression." *Id.*

Similarly, Shen *et al.* (Blood. 2004; 104(9):2936-9) (abstract attached as Exhibit 18) examined the level of B-cell lymphoma 2 (BCL2) protein expression in germinal center (GC) B-cells and diffuse large B-cell lymphoma (DLBCL). *Id.* at Abstract. The authors report that "GC cells had low expression commensurate with the low protein expression level" and that in DLBCL the level of BCL2 mRNA and protein expression showed "in general, a good correlation." *Id.*

Likewise, in an article by Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) the authors report that six mantle cell lymphomas studied "expressed either cyclin D2 (2 cases) or cyclin D3 (4 cases)." *Id.* at Abstract. "There was a good correlation between cyclin D protein expression and the corresponding mRNA expression levels by gene expression analysis." *Id.*

These examples are only a few of the many references Applicants could cite in rebuttal to the PTO's arguments. Applicants submit herewith 26 additional references (abstracts attached as Exhibit 20) which also support Applicants' assertion in that they report a correlation between the level of mRNA and corresponding protein, contrary to the assertion of the PTO that mRNA and protein levels are not correlated.

Applicants note that the new references submitted in the Information Disclosure Statement focus on the correlation between mRNA expression and protein expression levels, and for the most part do not examine gene amplification. However, those few references that actually looked at gene amplification **did** find a correlation between gene amplification and increased mRNA and protein expression levels.

For example, Bea *et al.* (Cancer Res. 2001; 61(6):2409-12) (abstract attached in Exhibit 12) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in human lymphoma samples. The authors found BMI-1 gene amplification in four mantle cell lymphomas (MCLs). Bea *et al.* report that "[t]he

four tumors with gene amplification showed significantly higher mRNA levels than other MCLs and NHLs with the BMI-1 gene in germline configuration" (Abstract; emphasis added). Applicants note that the fact that five additional MCLs also showed very high mRNA levels without gene amplification does not disprove Applicants' position, because one of skill in the art would understand that there can be more than one cause of mRNA overexpression. The issue is not whether mRNA overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression. Bea *et al.* further note that the four MCLS with gene amplification of *BMI-1* "showed significantly higher levels of mRNA **and protein expression** compared with other lymphomas with *BMI-1* in germline configuration" (page 2411, col. 1; emphasis added). Thus Bea *et al.* supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Godbout *et al.* (J. Biol. Chem. 1998; 273(33)21161-8) (abstract attached as Exhibit 14) studied the DEAD box gene, DDX1, in retinoblastoma and neuroblastoma tumor cell lines. The authors report that "**there is a good correlation with DDX1 gene copy number, DDX1 transcript levels, and DDX1 protein levels in all cell lines studied**" (Abstract). Thus Godbout *et al.* also supports Applicants' assertion that gene amplification is correlated with both increased mRNA and protein expression.

Applicants note that while Fu *et al.* (Blood 2005; 106(13):4315-21) (abstract attached as Exhibit 19) found increased mRNA and protein expression of cyclin D2 and cyclin D3 in the absence of gene amplification, this result proves only that increased mRNA and protein expression levels can result from causes other than gene amplification. As Applicants do not assert that gene amplification is the only cause of increased mRNA and protein expression levels, this result does not disprove Applicants assertion that that increased gene amplification, in general, is correlated with increased mRNA and protein expression.

In summary, Applicants submit herewith a total of 118 references in addition to the declarations and references already of record which support Applicants' asserted utility, either directly or indirectly. These references, together with the previous Orntoft, Hyman, Pollack and Hanna references of record, support the assertion that that in general, amplification of a particular gene leads to a corresponding change in the level of expression of the mRNA and encoded protein. These references further support the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions (*see, e.g.,* abstracts attached as Exhibit 21). However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See*

M.P.E.P. at §2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants' asserted utility. Applicants submit that considering the evidence as a whole, with the overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true."

Applicants therefore respectfully request withdrawal of the rejections of Claims 28-32 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned agent at the telephone number shown below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2830 P1C4**).

Respectfully submitted,

Date: March 9, 2007

By: _____
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